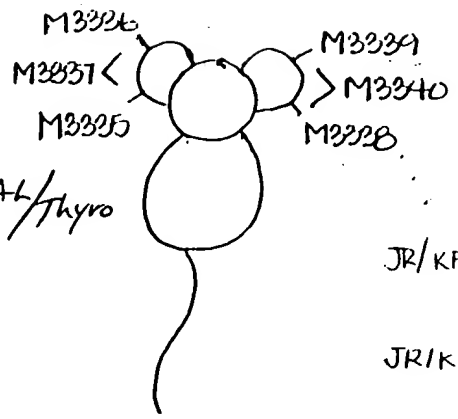
M3335-3340 N053



human iNOS CT: CR-NL-Om-SLEMSAL/Thyro

①	1ST I.P. Immunization & FCA	JR/KF	4/28/94
②	2nd IP Immunization w/ FCA	JR/KF	5/19/94
③	3rd IP Immunization	JR	6/9/94
④	Test bleed via orbital eye vein	JR	6/21/94
⑤	M3336, M3338: Final boost: 1/2 IV, 1/2 splenic/IP	JR/RW	6/27/94
⑥	M3336, M3338: Euth by cervical dislocation and spleens aseptically removed for fusion	JR	7/1/94
⑦	Test bleed via orbital eye vein	JR	9/29/94
⑧	4th: 1/2 IP, 1/2 intersplenic	JR/RW	9/29/94
⑨	Euth by cervical dislocation	RW	12/16/94

EASY BEAM

Plate # 1 Date 6 / 22 / 94 Filter 492 nm
Operator JC Comment N054 @ 100 ng/well
Test Bleed #1, IgG

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+1.066	+0.533	+0.226	+0.110	+0.062	+0.030	+0.014	+0.014	+0.005	+0.005	+0.000	A M3335
B	+0.000	+OVER	+OVER	+OVER	+1.923	+1.690	+1.166	+0.635	+0.325	+0.147	+0.073	+0.032	B M3336
C	+0.000	+1.461	+0.919	+0.392	+0.188	+0.091	+0.041	+0.019	+0.013	+0.007	+0.000	+0.005	C M3337
D	+0.000	+OVER	+OVER	+OVER	+1.879	+1.438	+0.822	+0.409	+0.184	+0.086	+0.044	+0.019	D M3338
E	+0.000	+OVER	+OVER	+1.984	+1.664	+1.117	+0.626	+0.313	+0.167	+0.080	+0.043	+0.020	E M3339
F	+0.000	+1.254	+0.876	+0.468	+0.252	+0.125	+0.063	+0.032	+0.017	+0.011	+0.008	+0.000	F M3340
G	======	======	======	======	======	======	======	======	======	======	======	======	G
H	======	======	======	======	======	======	======	======	======	======	======	======	H
	1	2	3	4	5	6	7	8	9	10	11	12	

Blank ↑ 1:100 ↑ 1:102400

EASY BEAM

Plate # 4 Date 6 / 22 / 94 Filter 492 nm
Operator JC Comment NO54 @ 100ng/well
Test Bleed #1, IgM

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.217	+0.131	+0.076	+0.041	+0.025	+0.013	+0.006	+0.006	+0.000	+0.000	+0.000	A M3335
B	+0.005	+0.568	+0.332	+0.175	+0.094	+0.049	+0.028	+0.013	+0.009	+0.000	+0.000	+0.000	B M3336
C	+0.000	+0.267	+0.180	+0.114	+0.059	+0.034	+0.019	+0.009	+0.000	+0.000	+0.000	+0.000	C M3337
D	+0.000	+0.382	+0.236	+0.124	+0.061	+0.031	+0.016	+0.010	+0.005	+0.000	+0.000	+0.005	D M3338
E	+0.000	+0.382	+0.227	+0.110	+0.055	+0.027	+0.015	+0.008	+0.000	+0.000	+0.000	+0.000	E M3339
F	+0.000	+0.227	+0.150	+0.096	+0.054	+0.035	+0.016	+0.008	+0.005	+0.000	+0.000	+0.000	F M3340
G	======	======	======	======	======	======	======	======	======	======	======	======	G
H	======	======	======	======	======	======	======	======	======	======	======	======	H
	1	2	3	4	5	6	7	8	9	10	11	12	

1 → Blank
2 → 1:100
12 → 1:102400

(Book 25)

SP2/0 : 20×T-75: Decanted supernatant and harvested cells in fresh media ; split flasks depending on confluency for fusion tomorrow.
Media = DME-w/ HEPES, 10% FCS, P/S

FGF-acidic : 1×T-25: Spun supernatant and harvested cells for 5' @ 1200rpm.
7.2×10^6c viable (~80%)
Reseeded T-25 @ 1×10^6c and removed 5.33×10^3 cells for cloning (20, 10, 5, 2.5, 1.25, .6)
Respun remaining cells (~6.2×10^6) and froze down @ ~2×10^6 c/ml (Media = DME-h, 20% FCS, 1% P/S, 10% DMSO)
Media = DME-h, 20% FCS, P/S

Ha

Friday, 1 July 1994

NO55 Fusion

SP2/0-Ag14 : 20×T-75: Decanted supernatant and harvested in fresh media (DME w/ HEPES, 10% FCS, P/S)
Split flasks 1:10 and collected cells in 4×T-75 for fusion. 2×T-75 counted: 1.7×10^7 vc (~88%)
2.0×10^7 vc (~92%)
Final count: 4.08×10^8 vc (~90%)

Splenocytes: 5min. in NH_4Cl sol.
Final count: 5.32×10^8 vc (~90%)

Fusion Ratio: 5.32×10^8 splenocytes : 4.08×10^8 SP2/0
1.25 : 1 ratio

Plating: Total # of cells: 9.4×10^8c → resuspend @ 2.5×10^6 c/ml
∴ 376ml (Resuspended in ~350ml and seeded 20×96well plates w/ 2 drops/well)
Media = DME-w/ HEPES, 20% FCS, 1x OH, P/S

EASY BEAM

Plate # N023-1 Date 1 / 14 / 94 Filter 492 nm
Operator JR Comment Sans @ 100ng/well w/ NO64
2° Ab = HRP-GAM IgG1/M cocktail

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.009	+0.000	+0.000	A
B	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.008	+0.005	B
C	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.006	+0.000	+0.000	+0.000	+0.007	+0.000	C
D	+0.000	+0.011	+0.000	-0.005	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.005	+0.005	D
E	-0.005	-0.005	+0.000	+0.000	+0.305	-0.006	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	E
F	-0.006	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	F
G	-0.005	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.009	+0.000	+0.000	+0.000	+0.005	G
H	+0.000	+0.005	+0.008	+0.000	+0.000	+0.000	+0.008	+0.013	+0.000	+0.000	+0.011	+0.011	H
	1	2	3	4	5	6	7	8	9	10	11	12	

IE5: 50-60% confluent w/ hybridomas, some fibroblasts

EASY BEAM

Plate # N053-2 Date 7 / 14 / 94 Filter 492 nm
Operator JR Comment ecns. w/ N054 @ 100ng/well
1°Ab = 20µl sup.
2°Ab = HRP-GAMIgG/M

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.008	+0.000	+0.000	+0.006	+0.011	+0.000	A
B	-0.009	+0.000	+0.000	-0.009	+0.000	-0.005	+0.000	+0.000	+0.000	+0.000	+0.008	+0.000	B
C	-0.011	+0.000	-0.010	-0.008	-0.008	+0.000	-0.007	+0.000	+0.000	+0.000	+0.000	+0.000	C
D	-0.007	+0.000	+0.000	+0.000	-0.007	-0.007	-0.005	+0.000	+0.000	+0.000	+0.008	+0.005	D
E	-0.010	+0.000	-0.005	-0.005	+0.000	+0.000	+0.000	-0.005	+0.009	+0.012	+0.000	+0.010	E
F	-0.011	+0.000	+0.000	+0.005	+0.007	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	F
G	-0.009	-0.007	-0.007	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.006	+0.000	G
H	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	H
	1	2	3	4	5	6	7	8	9	10	11	12	

EASY BEAM

Plate # NO83-3 Date 7 / 14 / 94 Filter 492 nm
Operator JR Comment 8cells w/ NO84 @ 100ng/well
1°Ab= 20µl sup.
2°Ab= HRP-GAMIgG/M

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.007	+0.000	+0.021	+0.005	+0.007	+0.009	+0.000	+0.010	+0.014	+0.010	+0.012	A
B	+0.000	+0.007	+0.000	+0.000	+0.009	+0.005	+0.007	+0.007	+0.007	+0.010	+0.013	+0.017	B
C	+0.000	+0.000	-0.008	+0.000	+0.000	+0.000	+0.000	+0.000	+0.022	+0.007	+0.018	+0.009	C
D	-0.005	+0.000	+0.000	+0.000	+0.000	+0.000	+0.006	+0.000	+0.000	+0.010	+0.010	+0.007	D
E	-0.007	+0.000	+0.000	+0.000	-0.008	+0.007	+0.016	+0.000	+0.005	+0.005	+0.005	+0.008	E
F	-0.007	+0.000	+0.000	+0.016	+0.000	+0.000	+0.000	+0.000	+0.000	+0.005	+0.008	+0.010	F
G	-0.005	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.005	+0.007	+0.007	G
H	+0.000	+0.000	+0.007	+0.007	+0.007	+0.007	+0.011	+0.005	+0.005	+0.005	+0.008	+0.011	H
	1	2	3	4	5	6	7	8	9	10	11	12	

EASY BEAM

Plate # N053-4 Date 1 / 14 / 94 Filter 492 nm
Operator JR Comment Bons w/ N054@100ng/well
1°Ab = 20µl ecip
2°Ab = HRP-GAMIgG/M

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.012	+0.000	+0.007	+0.007	+0.009	+0.005	+0.005	+0.005	+0.009	+0.011	+0.009	A
B	+0.000	+0.006	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.005	+0.011	+0.006	B
C	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.005	+0.000	C
D	+0.000	+0.000	+0.000	+0.000	+0.000	+0.008	+0.000	+0.000	+0.015	+0.000	+0.000	+0.000	D
E	-0.006	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	E
F	+0.009	+0.000	+0.000	+0.000	+0.000	+0.000	+0.005	+0.000	+0.000	+0.000	+0.000	+0.000	F
G	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	G
H	+0.000	+0.000	+0.006	+0.000	+0.000	+0.008	+0.010	+0.000	+0.000	+0.000	+0.007	+0.000	H
	1	2	3	4	5	6	7	8	9	10	11	12	

EASY BEAM

Plate # N053-5 Date 1 / 14 / 94 Filter 492 nm
Operator JR Comment BCDS.W/N054@100ng/well
1°Ab = 20µl Sup.
2°Ab = HRP-GAMIgG/M

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	-0.006	-0.031	+0.000	+0.000	+0.000	A
B	-0.005	+0.000	-0.005	+0.000	+0.000	+0.000	+0.000	+0.000	-0.030	+0.000	+0.000	+0.000	B
C	-0.011	-0.005	-0.011	+0.000	-0.009	-0.009	-0.006	-0.006	-0.031	+0.000	-0.005	+0.000	C
D	-0.013	-0.008	-0.010	-0.010	-0.010	-0.008	-0.006	-0.009	-0.031	+0.000	+0.000	+0.000	D
E	-0.015	-0.011	-0.011	-0.011	-0.011	-0.014	-0.012	-0.010	-0.030	-0.007	-0.005	+0.000	E
F	-0.010	-0.010	-0.006	+0.000	-0.008	-0.005	-0.007	-0.007	-0.032	+0.000	+0.000	+0.000	F
G	-0.011	-0.009	-0.011	-0.005	-0.009	-0.006	-0.006	-0.008	-0.031	+0.000	-0.005	+0.000	G
H	-0.006	+0.000	-0.005	+0.000	+0.000	+0.000	+0.000	+0.000	-0.029	+0.000	+0.000	+0.009	H
	1	2	3	4	5	6	7	8	9	10	11	12	

EASY BEAM

Plate # N053-6 Date 7 / 14 / 94 Filter 492 nm
Operator JR Comment Sons. w/ N084 @ 100ng/well
1°Ab = 20µl sup.
2°Ab = HRP-GAMIgG/M

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	-0.005	+0.000	+0.000	+0.000	-0.008	-0.008	-0.006	+0.000	+0.000	+0.000	+0.000	A
B	-0.006	-0.008	+0.000	-0.005	-0.007	-0.011	+0.000	+0.000	-0.007	+0.000	+0.000	+0.000	B
C	-0.010	+0.000	-0.013	-0.011	-0.017	-0.006	-0.010	-0.007	-0.007	+0.000	+0.000	+0.000	C
D	-0.010	-0.007	-0.007	-0.007	-0.009	-0.009	+0.000	+0.010	-0.010	-0.006	+0.000	+0.000	D
E	-0.009	-0.009	-0.018	-0.012	-0.014	-0.017	-0.012	-0.010	+0.000	-0.006	-0.006	-0.008	E
F	+0.000	-0.007	-0.007	-0.005	+0.000	-0.011	-0.011	-0.005	+0.000	+0.006	+0.029	-0.009	F
G	-0.009	-0.007	-0.011	-0.009	-0.005	-0.009	+0.000	-0.005	+0.000	-0.007	+0.000	+0.000	G
H	-0.009	-0.005	+0.000	+0.000	+0.000	+0.000	+0.023	+0.000	+0.000	+0.000	-0.005	+0.000	H
	1	2	3	4	5	6	7	8	9	10	11	12	

EASY BEAM

Plate # ND53-7 Date 7 / 14 / 94 Filter 492 nm
Operator JR Comment SOPS. W/ NOD1+@100ng/well
1°Ab = 20 µl Sup.
2°Ab = HRP-GAMIgG1/M

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.009	+0.005	+0.008	+0.006	+0.019	+0.007	+0.005	+0.009	+0.009	+0.009	+0.009	A
B	+0.015	+0.005	+0.007	+0.000	+0.000	+0.000	+0.006	+0.008	+0.011	+0.008	+0.008	+0.008	B
C	+0.011	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.011	+0.000	+0.007	+0.005	+0.007	C
D	+0.000	+0.000	+0.005	+0.000	+0.000	+0.000	+0.000	+0.005	+0.000	+0.029	+0.023	+0.009	D
E	+0.000	+0.000	+0.000	+0.006	+0.000	+0.000	+0.000	+0.000	+0.007	+0.000	+0.006	+0.961	E
F	+0.000	+0.000	+0.000	+0.005	+0.000	+0.000	+0.000	+0.000	+0.000	+0.045	+0.000	+0.000	F
G	+0.000	+0.000	+0.010	+0.000	+0.013	+0.000	+0.005	+0.000	+0.000	+0.009	+0.000	+0.005	G
H	+0.000	+0.000	+0.005	+0.000	+0.005	+0.005	+0.000	+0.000	+0.005	+0.008	+0.012	+0.009	H
	1	2	3	4	5	6	7	8	9	10	11	12	

7E12: 30-40% confluent, few fibroblasts

EASY BEAM

Plate # NOOB-8 Date 1 / 14 / 94 Filter 492 nm
Operator JR Comment 8005 W/ NYB4 @ 100ng/well
1°Ab = 20µl sup.
2°Ab = HRP-GAMIgG/M

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.000	+0.000	+0.060	+0.008	+0.000	+0.000	+0.000	+0.000	+0.000	+0.005	+0.000	A
B	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.009	B
C	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	C
D	-0.005	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.005	+0.000	D
E	+0.000	-0.007	+0.005	+0.000	+0.000	+0.000	+0.005	+0.000	+0.015	+0.000	+0.000	+0.000	E
F	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	F
G	+0.000	+0.000	+0.000	+0.000	+0.005	+0.000	+0.000	+0.000	+0.005	+0.005	+0.000	+0.007	G
H	+0.005	+0.000	+0.000	+0.000	+0.006	+0.006	+0.000	+0.000	+0.005	+0.005	+0.007	+0.005	H
	1	2	3	4	5	6	7	8	9	10	11	12	

8A4: 30% confluent, some fibroblasts

EASY BEAM

Plate # N083-9 Date 1 / 14 / 94 Filter 492 nm
Operator JR Comment BCAS w/ N084 @ 100ng/well
1°Ab = 20µl Sup.
2°Ab = HRP-GAMIgG/M

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.005	+0.000	+0.005	+0.005	+0.000	+0.006	+0.000	+0.005	+0.012	+0.012	+0.007	A
B	+0.000	+0.000	+0.000	+0.006	+0.000	+0.000	+0.000	+0.000	+0.000	+0.009	+0.009	+0.009	B
C	+0.000	+0.000	-0.010	+0.000	-0.007	-0.005	+0.000	+0.000	+0.006	+0.006	+0.009	+0.018	C
D	+0.000	+0.000	+0.017	-0.006	-0.006	+0.000	+0.000	+0.000	+0.000	+0.006	+0.012	+0.008	D
E	+0.000	+0.000	-0.009	-0.006	-0.008	+0.000	-0.006	+0.000	+0.006	+0.006	+0.012	+0.016	E
F	-0.006	+0.006	+0.000	+0.006	+0.000	+0.000	-0.006	+0.008	+0.005	+0.007	+0.005	+0.005	F
G	+0.005	+0.020	+0.000	+0.012	-0.005	+0.005	+0.000	+0.014	+0.005	+0.007	+0.007	+0.000	G
H	+0.000	+0.000	+0.010	+0.007	+0.145	+0.010	+0.006	+0.008	+0.005	+0.009	+0.009	+0.009	H
	1	2	3	4	5	6	7	8	9	10	11	12	

9HB: 90% confluent

EASY BEAM

Plate # N053-10 Date 1 / 14 / 94 Filter 492 nm
Operator JR Comment 8cns. w/ N004 @ 100ng/well
1°Ab-20µl sup.
2°Ab=HRP-GAMIgG/M

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.009	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.005	+0.000	+0.025	A
B	+0.000	+0.000	+0.000	+0.000	+0.005	+0.000	+0.000	+0.000	+0.000	+0.005	+0.000	+0.000	B
C	+0.000	+0.000	-0.005	+0.000	+0.000	+0.000	-0.007	-0.005	-0.005	+0.000	+0.000	+0.000	C
D	-0.006	+0.000	+0.000	-0.005	+0.000	+0.000	+0.000	+0.000	+0.000	+0.007	+0.000	+0.000	D
E	+0.000	+0.000	+0.000	-0.005	+0.000	+0.000	-0.006	+0.000	+0.009	+0.000	+0.000	+0.000	E
F	+0.000	+0.000	+0.000	+0.000	+0.000	-0.006	-0.006	+0.000	+0.005	+0.000	+0.000	+0.000	F
G	+0.007	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	G
H	+0.000	+0.000	+0.000	+0.000	+0.006	+0.008	+0.000	+0.000	+0.006	+0.000	+0.011	+0.008	H
	1	2	3	4	5	6	7	8	9	10	11	12	

EASY BEAM

Plate # N083-11 Date 7 / 14 / 94 Filter 492 nm
Operator JR Comment Cons. w/ N064 @ 100ng/well
1°Ab = 20µl sup.
2°Ab = HRP-GAMIgG/M

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.000	+0.000	-0.005	+0.000	+0.007	+0.000	-0.005	+0.000	+0.000	+0.000	+0.000	A
B	+0.000	+0.006	+0.000	+0.000	+0.000	-0.005	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	B
C	+0.000	+0.005	+0.000	-0.007	-0.007	+0.000	-0.005	+0.000	-0.007	+0.000	+0.000	+0.000	C
D	+0.000	+0.000	+0.000	+0.000	+0.000	-0.007	-0.005	+0.000	+0.000	+0.000	+0.000	-0.005	D
E	+0.052	+0.000	+0.000	+0.000	-0.005	+0.000	+0.000	-0.007	-0.007	-0.007	-0.005	-0.005	E
F	+0.000	+0.000	+0.042	+0.015	+0.015	-0.005	-0.009	-0.005	-0.005	-0.005	-0.005	-0.005	F
G	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	-0.005	-0.005	-0.010	+0.000	+0.000	+0.000	G
H	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	H
	1	2	3	4	5	6	7	8	9	10	11	12	

IIE1: 40% confluent, some fibroblasts

IIF3: 60-70% confluent, few fibroblasts

EASY BEAM

Plate # NOS3-12 Date 1 / 14 / 94 Filter 492 nm
Operator JR Comment SONS. W/ NOS4-B 100ng/well
1°Ab = 20µl SUP.
2°Ab = HRP-GAMIgG/M

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.005	+0.000	+0.000	+0.027	-0.005	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	A
B	+0.000	+0.000	+0.005	+0.000	+0.000	-0.006	+0.000	+0.000	+0.000	-0.005	+0.000	+0.000	B
C	+0.000	+0.000	+0.000	-0.008	-0.005	-0.007	-0.007	+0.000	-0.005	-0.005	+0.023	+0.000	C
D	-0.006	+0.000	+0.148	-0.012	+0.000	-0.007	-0.007	-0.007	+0.000	+0.007	+0.000	+0.000	D
E	-0.005	-0.013	+0.000	+0.000	-0.006	-0.010	-0.007	-0.007	+0.000	-0.007	+0.000	-0.008	E
F	+0.000	+0.000	-0.007	-0.007	+0.000	-0.010	-0.006	+0.000	-0.007	+0.000	-0.007	+0.000	F
G	+0.000	-0.010	-0.008	+0.013	-0.007	-0.011	+0.000	+0.000	+0.000	+0.000	+0.000	-0.005	G
H	+0.000	+0.000	+0.017	-0.005	-0.005	-0.008	-0.008	+0.000	+0.000	+0.000	+0.000	+0.000	H
	1	2	3	4	5	6	7	8	9	10	11	12	

12D3: 95% confluent

EASY BEAM

Plate # N053-13 Date 1 / 14 / 94 Filter 492 nm
Operator JR Comment Sens. w/ N054 @ 100ng/well
1°Ab = 20 µl sup.
2°Ab = HRP-GAMIgG/M

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.006	+0.006	A
B	+0.000	+0.000	+0.006	+0.006	+0.000	+0.000	+0.000	+0.007	+0.005	+0.000	+0.047	+0.005	B
C	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.014	+0.006	+0.000	+0.009	+0.009	C
D	+0.000	+0.000	+0.006	+0.000	+0.006	+0.006	+0.006	+0.006	+0.006	+0.000	+0.009	+0.006	D
E	+0.000	+0.000	+0.000	+0.000	+0.000	+0.005	+0.007	+0.000	+0.000	+0.005	+0.005	+0.005	E
F	+0.005	+0.005	+0.005	+0.000	+0.006	+0.006	+0.000	+0.005	+0.000	+0.010	+0.007	+0.007	F
G	+0.005	+0.000	+0.005	+0.030	+0.010	+0.000	+0.005	+0.005	+0.005	+0.007	+0.007	+0.007	G
H	+0.000	+0.000	+0.007	+0.010	+0.008	+0.005	+0.000	+0.007	+0.028	+0.009	+0.035	+0.011	H
	1	2	3	4	5	6	7	8	9	10	11	12	

13B11: 60% confluent, few fibroblasts

EASY BEAM

Plate # N003-14 Date 1 / 14 / 94 Filter 492 nm
Operator JR Comment 80ng. w/ N004 @ 100ng/well
1°Ab = 20µl sup.
2°Ab = HRP-GAMIgG/M

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.000	+0.000	+0.000	+0.000	+0.005	+0.007	+0.007	+0.007	+0.011	+0.008	+0.008	A
B	+0.016	+0.007	+0.007	+0.007	+0.007	+0.010	+0.010	+0.010	+0.006	+0.019	+0.010	+0.010	B
C	+0.008	+0.000	+0.005	+0.005	+0.008	+0.005	+0.007	+0.010	+0.007	+0.014	+0.043	+0.009	C
D	+0.007	+0.007	+0.007	+0.016	+0.006	+0.008	+0.008	+0.008	+0.008	+0.011	+0.011	+0.009	D
E	+0.000	+0.000	+0.006	+0.008	+0.011	+0.005	+0.009	+0.009	+0.005	+0.010	+0.012	+0.012	E
F	+0.000	+0.005	+0.008	+0.010	+0.005	+0.005	+0.007	+0.007	+0.011	+0.011	+0.011	+0.011	F
G	+0.000	+0.000	+0.005	+0.022	+0.007	+0.007	+0.007	+0.011	+0.009	+0.006	+0.012	+0.009	G
H	+0.007	+0.007	+0.007	+0.007	+0.005	+0.011	+0.007	+0.011	+0.013	+0.011	+0.013	+0.007	H
	1	2	3	4	5	6	7	8	9	10	11	12	

EASY BEAM

Plate # N003-16 Date 1 / 14 / 94 Filter 492 nm
Operator JR Comment SOS.w/NOS+@100ng/well
1°Ab=20µl sup.
2°Ab-HRP-GAMIgG/M

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.000	+0.006	+0.000	+0.000	+0.000	+0.000	+0.005	+0.014	+0.027	+0.000	+0.019	A
B	+0.000	+0.000	+0.021	+0.000	+0.005	+0.000	+0.005	+0.000	+0.005	+0.000	+0.021	+0.000	B
C	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.005	+0.005	C
D	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.006	+0.000	D
E	+0.000	+0.000	+0.005	+0.005	+0.000	+0.000	+0.000	+0.000	+0.000	+0.010	+0.000	+0.009	E
F	+0.000	+0.005	+0.005	+0.000	+0.000	+0.000	+0.000	+0.006	+0.006	+0.000	+0.000	+0.000	F
G	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.009	+0.000	+0.015	+0.000	+0.000	+0.000	G
H	+0.000	+0.000	+0.005	+0.011	+0.000	+0.007	+0.010	+0.019	+0.000	+0.000	+0.006	+0.000	H
	1	2	3	4	5	6	7	8	9	10	11	12	

EASY BEAM

Plate # N033-16 Date 7 / 14 / 94 Filter 492 nm
Operator JR Comment 8CHS. w/ NO5+ @ 100ng/well
1°Ab = 20µl sup.
2°Ab = HRP-GAMIgG/M

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.000	+0.008	+0.000	+0.000	+0.005	+0.005	+0.000	+0.000	+0.009	+0.000	+0.000	A
B	+0.000	+0.006	+0.006	+0.000	+0.015	+0.006	+0.006	+0.006	+0.006	+0.008	+0.008	+0.006	B
C	+0.000	+0.009	+0.000	+0.000	+0.012	+0.000	+0.000	+0.000	+0.006	+0.006	+0.000	+0.059	C
D	+0.009	+0.005	+0.005	+0.000	+0.007	+0.000	+0.006	+0.006	+0.006	+0.000	+0.000	+0.000	D
E	+0.006	+0.000	+0.000	+0.000	+0.000	+0.008	+0.000	+0.007	+0.005	+0.000	+0.000	+0.000	E
F	+0.007	+0.007	+0.020	+0.005	+0.000	+0.000	+0.005	+0.005	+0.000	+0.000	+0.005	+0.000	F
G	+0.005	+0.012	+0.009	+0.019	+0.005	+0.018	+0.005	+0.005	+0.000	+0.005	+0.009	+0.000	G
H	+0.013	+0.007	+0.007	+0.013	+0.009	+0.006	+0.000	+0.000	+0.005	+0.010	+0.007	+0.000	H
	1	2	3	4	5	6	7	8	9	10	11	12	

16C12: 40-50% confluent, few fibroblasts

EASY BEAM

Plate # N003-17 Date 1 / 14 / 94 Filter 492 nm
Operator JR Comment Scns. w/ N004+@100ng/well
1°Ab = 20µl sup.
2°Ab = HRP-GAMIgG/M

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.044	+0.000	-0.006	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	A
B	+0.000	+0.000	+0.000	+0.008	+0.008	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	B
C	+0.006	-0.006	+0.000	+0.000	+0.000	-0.006	-0.006	+0.000	+0.000	+0.000	+0.000	+0.000	C
D	+0.000	+0.000	+0.006	+0.000	-0.005	+0.000	+0.478	+0.000	+0.000	+0.000	+0.000	+0.000	D
E	+0.000	-0.006	-0.006	+0.000	+0.000	-0.005	+0.000	+0.000	+0.000	+0.000	+0.000	-0.005	E
F	+0.000	+0.000	+0.005	+0.000	+0.000	+0.000	+0.000	+0.000	-0.005	+0.000	-0.005	+0.000	F
G	+0.000	-0.005	+0.000	+0.000	+0.000	-0.005	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	G
H	+0.000	+0.000	+0.000	+0.009	+0.000	+0.000	+0.000	+0.018	+0.000	+0.000	+0.000	+0.000	H
	1	2	3	4	5	6	7	8	9	10	11	12	

17A1: 100% confluent
17D7: 80-90% confluent, some fibroblasts

EASY BEAM

Plate # N053-18 Date 7 / 14 / 94 Filter 492 nm
Operator JR Comment Sons. w/ N054 @ 100ng/well
1°Ab ~20µl sup.
2°Ab=HRP-GAMIgG/M

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+1.203	+0.038	+0.065	+0.000	+0.020	+0.029	+0.000	+0.029	+0.018	+0.018	+0.009	+0.005	A
B	+0.045	+0.014	+0.141	+0.190	+0.131	+0.103	+0.125	+0.021	+0.033	+0.008	+0.000	+0.000	B
C	+0.000	+0.015	+0.000	+0.011	+0.178	+0.088	+0.011	+0.011	+0.021	+0.021	+0.000	+0.016	C
D	+0.045	+0.018	+0.005	+0.013	+0.008	+0.000	+0.043	+0.069	+0.005	+0.000	+0.000	+0.000	D
E	+0.039	+0.000	+0.020	+0.145	+0.118	+0.106	+0.047	+0.000	+0.000	+0.059	+0.012	+0.000	E
F	+0.067	+0.192	+0.000	+0.013	+0.013	+0.005	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	F
G	+0.000	+0.110	+0.000	+0.000	+0.000	+0.183	+0.036	+0.017	+0.102	+0.000	-0.006	+0.013	G
H	+0.000	+0.126	+0.075	+0.309	+0.026	+0.059	+0.134	+0.005	+0.186	+0.035	+0.007	+0.035	H
	1	2	3	4	5	6	7	8	9	10	11	12	

18A1 no hybrids ; fibroblasts
A3 " "
B1 90%. ; few
B3 70%.
B4 50%.
B5 20%. ; some
B6 80-90%
B7 80%.
C5 no hybrids ; fibroblasts
C6 30-40%. ; few
D1 10-20%. ; fibroblasts
D7 80%. ; few
D8 30%. ; some

18E4 very small colony ; fibroblasts
E5 5-10%. ; fibroblasts
E6 5-10% ; fibroblasts
E7 20%. ; some
E10 40% ; fibroblasts
F1 10-20%. ; fibroblasts
F2 70% ; some
G6 50%.
G9 no hybrids ; some fibro.
H2 " "
H3 couple small colonies ; fibro.
H4 no hybrids ; some fibro.
H6 50-60%. ; few
H7 60-70%. ; few
H9 20% ; few

EASY BEAM

Plate # N053-19 Date 1 / 14 / 94 Filter 492 nm
Operator JR Comment Sons. w/ N084 @ 100ng/well
1°Ab = 20µl sup.
2°Ab = HRP-GAMIgG/M

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.663	+0.012	+0.092	+0.012	+0.020	+0.342	+0.360	+0.242	+0.028	+0.919	+1.623	+0.008	A
B	+0.373	+0.049	+0.103	+0.427	+0.010	+0.007	+0.017	+0.011	+0.025	+0.007	+0.007	+0.030	B
C	+0.049	+0.006	+0.006	+0.019	+0.000	+0.007	+0.007	+0.007	+0.007	+0.000	+0.000	+0.008	C
D	+0.000	+0.009	+0.009	+0.000	+0.000	+0.000	+0.006	-0.005	+0.007	+0.007	+0.007	+0.007	D
E	+0.000	+0.000	+0.000	+0.014	+0.000	+0.000	+0.009	+0.005	+0.011	+0.005	+0.005	+0.007	E
F	+0.090	+0.019	+0.007	+0.007	+0.000	+0.008	+0.014	+0.014	+0.000	+0.005	+0.010	+0.000	F
G	+0.091	+0.005	+0.000	+0.000	+0.000	+0.000	+0.010	+0.005	+0.009	+0.018	+0.018	+0.000	G
H	+0.000	+0.008	+0.006	+0.006	+0.000	+0.018	+0.007	+0.007	+0.005	+0.000	+0.000	+0.007	H
	1	2	3	4	5	6	7	8	9	10	11	12	

19A1 : 40%. ; fibroblasts
A3 small colony ; fibro.
A6 60%.
A7 40%. ; fibro.
A8 30%. ; fibro.
A10 very small colony ; some
A11 100%
B1 70%
B2 10%? ; fibro.
B3 50% ; some
B4 small colony ; fibro.

19C1 10-20%.
F1 30% ; few
G1 no hybrids ; some

EASY BEAM

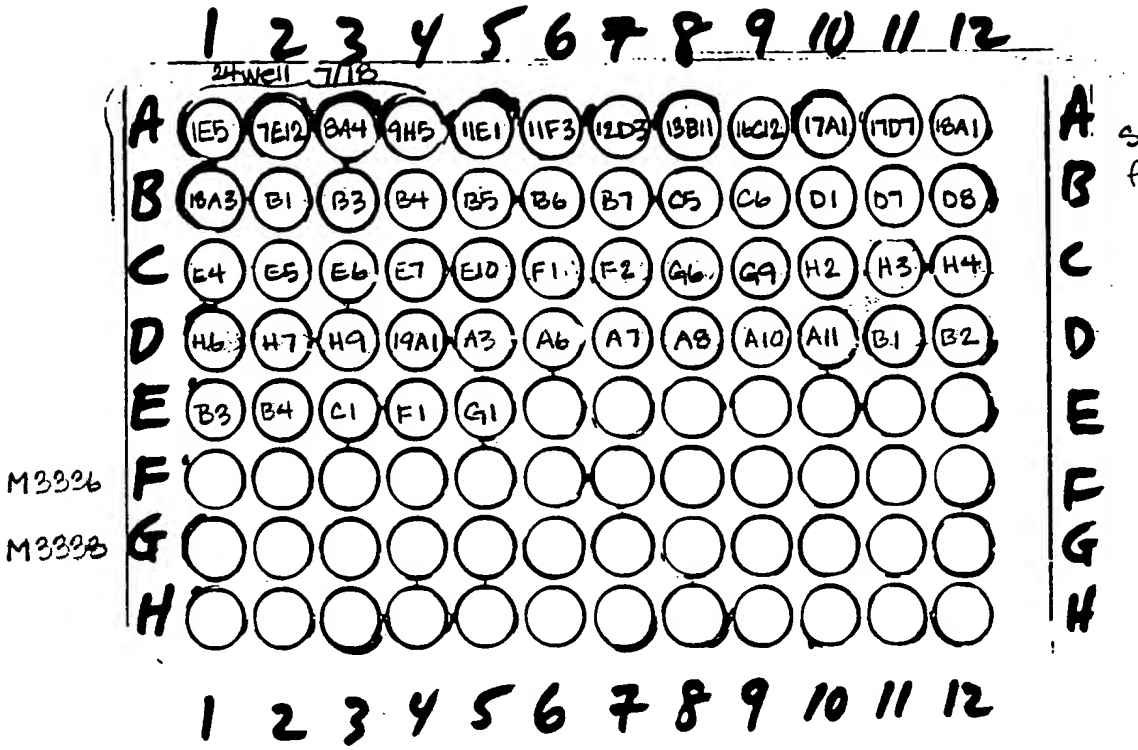
Plate # N053-20 Date 1 / 14 / 94 Filter 492 nm
Operator JR Comment Sens. w/ N53+ @ 100ng/well
1°Ab = 20µl 8up.
2°Ab = HRP-GAMIgG/M

	1	2	3	4	5	6	7	8	9	10	11	12	
A	-0.006	+0.012	+0.000	-0.009	+0.000	+0.000	+0.000	+0.000	-0.006	-0.006	+0.000	+0.000	A
B	+0.000	+0.000	+0.000	+0.000	+0.000	-0.005	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	B
C	-0.006	+0.000	-0.006	-0.006	-0.006	+0.000	+0.000	+0.000	+0.000	-0.006	-0.006	+0.000	C
D	+0.000	+0.000	+0.015	+0.000	+0.000	-0.006	+0.000	+0.000	+0.005	+0.000	+0.000	+0.006	D
E	-0.007	+0.000	-0.006	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	E
F	-0.013	+0.007	+0.007	+0.000	+0.010	+0.010	+0.010	+0.008	+0.010	+0.008	+0.000	+0.011	F
G (13336)	Blank -0.011	1:100 +0.629	+0.296	+0.150	+0.093	1:1600 +0.063	Blank -0.017	1:100 +1.830	+1.717	+1.489	+1.287	1:1600 +1.106	G
H (13338)	-0.011	+1.081	+0.690	+0.345	+0.205	+0.096	-0.011	+OVER	+OVER	+1.946	+1.830	+1.657	H
	1	2	3	4	5	6	7	8	9	10	11	12	

M

G

NOD's ELISA screen
100 µl supernatant
7/18/94



EASY BEAM

Plate # N053 Date 1 / 19 / 94 Filter 492 nm
Operator JR Comment Sons. w/ N054 @ 100ng/well
2°Ab = HRP-GAMIgG

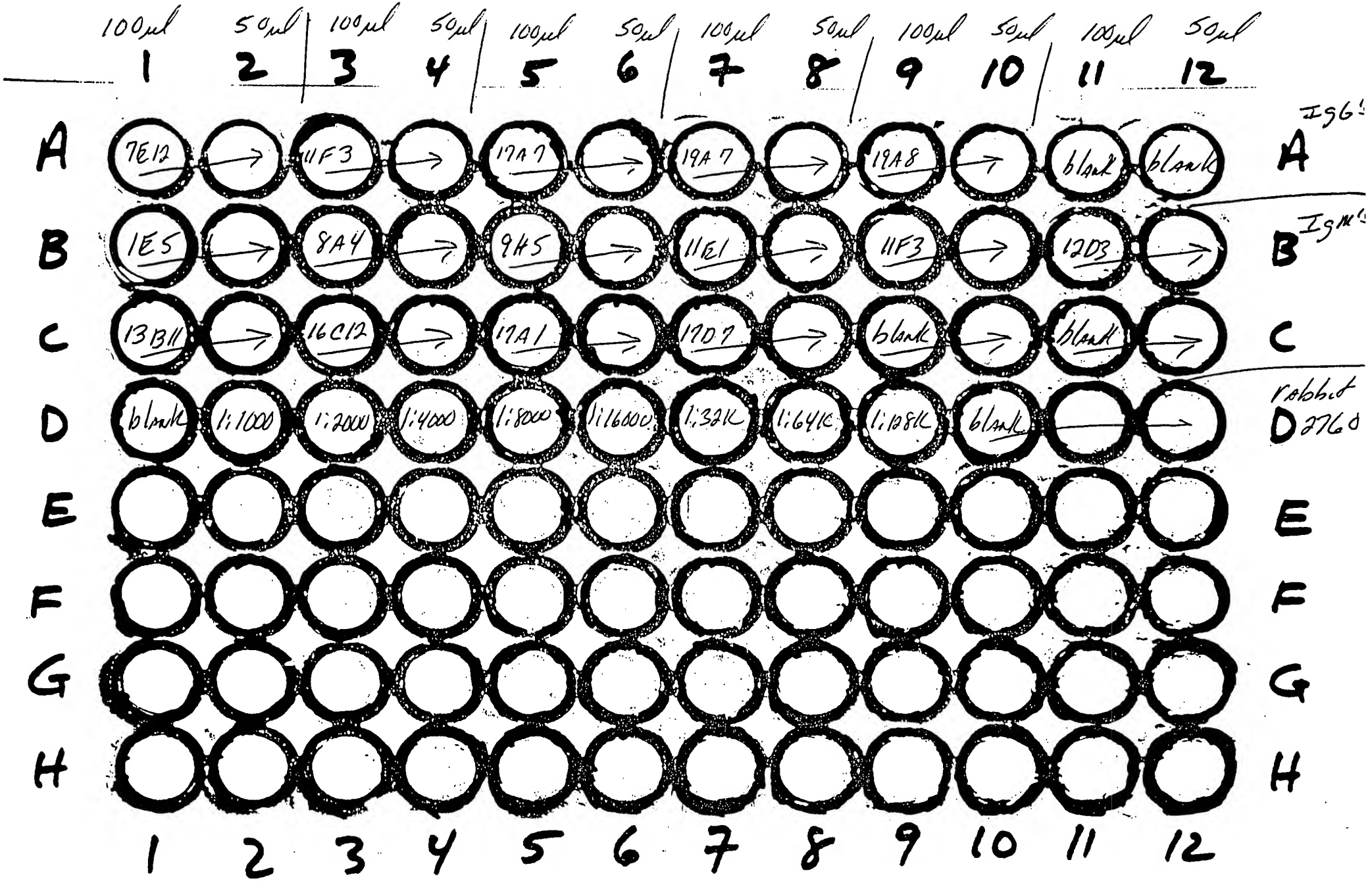
	1	2	3	4	5	6	7	8	9	10	11	12	
		7E12				11F3					17D7		
A	+0.000	+1.946 100µl	+0.000	+0.000	+0.000	+0.029 100µl	+0.000	-0.006	-0.006	+0.000	+0.177 100µl	-0.007	A
B	-0.007	+0.000	-0.005	+0.000	-0.005	+0.000	+0.000	-0.008	-0.006	-0.006	-0.006	+0.000	B
C	-0.007	-0.007	-0.005	+0.000	+0.000	-0.006	+0.000	+0.000	-0.006	+0.000	+0.000	-0.005	C
							19A7	19A8					
D	+0.000	+0.000	+0.013	+0.013	+0.007	+0.000	+0.089 50µl	+0.046 50µl	+0.005	+0.000	+0.000	+0.000	D
E	-0.005	-0.009	-0.007	+0.000	-0.006	+0.000	-0.006	+0.000	+0.000	+0.000	+0.000	+0.000	E
		Blank	1:100										
M3334 F	+0.000	+OVER	+1.984	+1.946	+1.879	+1.778	+1.525	+1.063	+0.637	+0.331	+0.175	+0.084	F
M3338 G	+0.000	+OVER	+OVER	+1.958	+1.923	+1.849	+1.762	+1.563	+1.187	+0.700	+0.374	+0.179	G
H	======	======	======	======	======	======	======	======	======	======	======	======	H
	1	2	3	4	5	6	7	8	9	10	11	12	

EASY BEAM

Plate # N053 Date 1 / 19 / 94 Filter 492 nm
Operator JR Comment Sons. w/ N054 @ room/well
2°Ab = HRP-GAMIgM

	1	2	3	4	5	6	7	8	9	10	11	12	
	1E5		8A4	9H5	11E1	11F3	12D3	13B11	16C12	17A1	17D7		
A	+0.124 100µl	-0.007	+0.041 100µl	+0.301 100µl	+0.219 100µl	+0.146 100µl	+0.120 100µl	+0.203 100µl	+0.980 100µl	+0.218 100µl	+1.046 100µl	-0.007	A
B	-0.007	-0.007	+0.000	+0.000	-0.006	-0.006	-0.006	-0.006	-0.006	+0.000	+0.017	-0.005	B
C	-0.009	-0.009	-0.009	-0.009	-0.009	-0.009	-0.007	-0.007	-0.007	-0.007	-0.007	-0.007	C
D	-0.007	-0.005	-0.008	-0.005	-0.008	+0.000	+0.000	-0.007	-0.007	+0.000	-0.005	-0.005	D
E	-0.008	-0.010	-0.010	-0.010	-0.010	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	E
F (M3336)	Blank +0.000	1:100 +1.121	+0.818	+0.468	+0.162	+0.059	+0.030	+0.018	+0.010	+0.007	+0.000	+0.000	F
G (M3336)	+0.000	+1.347	+1.168	+0.874	+0.496	+0.182	+0.051	+0.017	+0.005	+0.000	+0.000	+0.000	G
H	======	======	======	======	======	======	======	======	======	======	======	======	H
	1	2	3	4	5	6	7	8	9	10	11	12	

Testing Positive Hybridomas for binding 7-22-94
to rhiNOS (8-19-93 batch) @ 100µl + 50µl culture supernatant



EASY BEAM

Plate # 1 Date 7/23/94 Filter 492 nm
Operator DF Comment rhiNOSe 10ng/well (8/19/93 batch)

	100µl	50µl	100µl	50µl	100µl	50µl	100µl	50µl	100µl	50µl	100µl	50µl	
	1	2	3	4	5	6	7	8	9	10	11	12	
A	7E12 →		11F3 →		17D? →		19A1 →		19A8 →		blank →		A
	+0.010	+0.000	+0.000	-0.006	+0.006	+0.000	-0.007	+0.000	+0.000	+0.000	+0.005	+0.000	
B	1E5 →		8A4 →		9H5 →		11E1 →		11F3 →		12D3 →		B
	+0.000	+0.000	+0.000	+0.000	+0.000	+0.014	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	
C	13B11 →		16C12 →		12A1 →		17D ? →		blank →		blank →		C
	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.005	+0.000	+0.000	
D	blank	1:1000	1:2000	1:4000	1:8000	1:16K	1:32K	1:64K	1:128K	blanks →			D
	+0.009	+1.341	+1.114	+0.714	+0.658	+0.390	+0.214	+0.130	+0.009	+0.000	+0.005	+0.000	
E	======	======	======	======	======	======	======	======	======	======	======	======	E
F	======	======	======	======	======	======	======	======	======	======	======	======	F
G	======	======	======	======	======	======	======	======	======	======	======	======	G
H	======	======	======	======	======	======	======	======	======	======	======	======	H
	1	2	3	4	5	6	7	8	9	10	11	12	

IgG's

IgM's

Rabbit 2760

NO MAb Bindings
to rhiNOS whole protein